provide a quantitative description of the positive and negative ions that surround the nucleosome. Results of these experiments will be presented. This work should have implications for nucleosome compaction, chromatin remodeling, and more generally electrostatics of highly charged biomolecules.

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Stress-Activated Sliding Motion: A Coupled-Potential Model
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In a notable study by Julicher & Bruinsma (BPJ 74, 1998), a model was presented describing the motion of an RNAP molecule during an elongation cycle. First, stepping motion of a catalytic (C)-site takes place. This generates stress in the molecule between this (C)-site and a front (F)-site. This stress activates forward sliding of the (F)-site by lowering the activation barrier hindering its motion.
Here we look at this RNAP model in terms of a coupled-potential paradigm, taken from an inchworm-like model of a polymer chain. This model describes how stress produced by forward motion of a (C)-like site in the polymer leads to activated sliding of an (F)-like site (Joseph, J Polymer Sci 16, 1978). The (F)-like site has two positions - (1) and (2) - and so possesses a double-well potential. This site is coupled in series to a linear spring - with a single-well harmonic potential. A (C)-like site occupies the spring free end.
We find that coupling (adding) these two potentials yields a net potential with the (F)-like site occupying position (1). Also, there is a large activation barrier hindering movement to position (2). However, when the (C)-like site at the spring free end is pulled forward, the stretching of the spring causes the equilibrium position of the harmonic potential minimum to be shifted forward. Coupling of this shifted potential reduces the net potential barrier, and this reduction activates forward sliding motion of the (F)-like site.
We conclude that this type of coupled-potential inchworm model yields insight into how stress can activate sliding motion during the RNAP elongation cycle.

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High Resolution Surface Plasmon Microscopy: From Nano-colloids To Single Nucleosome Imaging
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Surface plasmon resonance (SPR) has been widely recognized as a highly sensitive and non intrusive method for probing modifications of adsorbed layers, and in particular for its application to characterize biomolecular specific interactions such as antigen-antibody recognition.
We apply SPR to study nucleosomes, first level of DNA compaction around an octamer of proteins called histones. To maintain the DNA helix accessible to the transcription and replication machineries during the cell cycle, this complex is highly dynamical (formation, disassembly, or sliding of the nucleosome), leading to rapid modifications of the whole chromatin structure *in vivo*. We aim at understanding how the DNA sequence influences the structure and dynamics of the nucleosomes in chromosomes.
The scanning surface plasmon microscope (SSPM) set-up relies on the use of a high numerical aperture objective that confines the surface plasmon polaritons (SPPs) to an area of the interface much smaller (up to a few hundreds of nanometers) than their typical propagation length (few microns). Similarly to the Kretschmann configuration, SPPs are excited at the resonance angle θ_P. As an objective is used to focus the purely P-polarized (radial) light, the SPPs converge to the center of the illuminated area, leading to the creation of SPPs interferences that will reradiate in symmetrical rays and go through the objective to be detected. The presence of non-marked biological samples at the interface modifies the propagation conditions (i.e. the interferences) of the SPPs, leading to a change in the image contrast. The SSPM point spread function is about 150 nm in aqueous medium, providing high resolution images of biological samples *in vitro*.
We will present the SSPM study of the optical response of gold and latex nanoparticles and then the first images of non-marked single nucleosomes in liquid medium.

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Ion Exchange in the Nonspecific Bimolecular Association and the Unimolecular DNA Bending in Specific Binding of IHF to DNA
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Protein-DNA interactions are strongly modulated by salt. Previous studies on the equilibrium binding of a cognate DNA sequence H′ to integration host factor (IHF), an architectural protein from *E. coli* that bends its cognate site by nearly 180°, have shown that the slope SK_D of ~8 on a log(K_D) versus log([KCl]) plot depends on the anion type, suggesting that both release of counterions from the DNA and the uptake and release of ions from the protein must be playing a role. Here, we probe the effect of [KCl] on the bimolecular association/dissociation as well as the unimolecular bending/unbending rates, by monitoring the relaxation kinetics of the complex between IHF and ~35-bp long H' substrate end-labeled with a FRET pair, in response to a laser temperature-jump. Our results and analysis reveal two notable results. First, that the unimolecular bending step is nearly independent of [KCl]. Second, that the bulk of the salt-dependence appears in the nonspecific association/dissociation step, with the equilibrium constant for that step accounting for more than half of the total SK_D observed. The latter result is in contrast to what one might expect if counterion release from the DNA was the dominant contribution to the salt-dependence, since the extent to which the H' substrate makes contact with the protein in the fully wrapped specific complex is significantly greater than in the nonspecific complex. One possible scenario is that counterion release is the dominant term in the formation of the nonspecific complex, whereas in the transition from the nonspecific to the specific complex, the extent of the counterion release is masked by the uptake and release of ions by the protein, as a result of conformational rearrangements in the protein.

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Analysis of RPA70N Involvement in RPA ssDNA Binding Activity
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RPA is the primary single-stranded DNA (ssDNA) binding protein in eukaryotes. It plays a central role in chromosomal DNA replication, repair and recombination pathways, protecting ssDNA from degradation by nucleases. RPA also mediates interactions with specific proteins active in these various DNA processing events. RPA has three subunits, each named after its molecular weight: RPA70 (domains N, A, B and C), RPA32 (domains N, D and C) and RPA14 (single domain). The N-terminal domain of RPA70 (70N) is flexibly linked by an 80 amino acid linker to the rest of RPA 70. It has long been established that RPA binds ssDNA with nM affinity through the action of domains 70A, 70B, 70C and 32D using 3 modes of binding. The first mode involves 70A and 70B spanning 8 nucleotides, the second mode adds 70C and spans 18 to 20 nucleotides, and the third mode adds 32D and spans 28-30 nucleotides. Tandem DNA binding by domains 70A and 70B is required for high affinity. Recently, a proposal has been made that 70N contributes to DNA binding function. However, 70N binding affinity is more than 1000-fold weaker than RPA70AB and all evidence shows 70N is primarily a protein-protein interaction domain targeting transcription factors and checkpoint proteins. This study aims to resolve this controversy by analyzing the effect of 70N on the ssDNA binding activity of the high affinity RPA DNA binding domains, 70A and 70B. We propose the use of size exclusion chromatography and isothermal titration calorimetry (ITC) to do a systematic comparison of the DNA binding properties of 70AB versus the 70NAB construct. We expect to show that 70AB DNA binding affinity is the same as 70NAB. This will provide conclusive evidence that the 70N domain is not involved in binding ssDNA.

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The Role of DNA "Bendability" in the Indirect Read-Out Mechanism of Protein-DNA Interactions
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Integration host factor (IHF) from *E. coli* is a DNA-bending protein that recognizes and binds to its specific sites primarily by the indirect read-out mechanism, in which sequence-dependent DNA dynamics and flexibility play an important role. The crystal structure of IHF bound to a 35-bp long cognate site H' indicates that the DNA is kinked at two sites separated by ~9 bp, resulting in a ''U-turn'' bend of the DNA. We use laser temperature-jump to perturb the IHF-DNA complex, and time-resolved FRET on end-labeled DNA substrates to monitor the bending/unbending dynamics. In our previous studies, we suggested that spontaneous DNA bending from transient disruption of base-pairing and/or stacking interactions at the site of the kinks may be the rate-limiting step in the transition from the nonspecific to the specific complex. Here, we investigate DNA bending kinetics for substrates with mismatched pairs introduced at the site of the kinks. These internal ''loops'' are expected to decrease the energetic cost of bending the DNA, which is reflected in the >10-fold increase in the binding affinity. Kinetics measurements on IHF bound to such DNA reveal